bio-protocol Preprint

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Crystal violet biomass assays

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An abbreviated version of this protocol was published in eLIFE in Mar 2022

Impact of a human gut microbe on *Vibrio cholerae* host colonization through biofilm enhancement

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Detailed protocol

Outlined below is our crystal violet staining assay, based off the O'Toole 2011 protocol (PMID 21307833).

1. Incubate samples in a 96-well plate for the desired time.
    - For our experiments, we used tissue-culture treated plates and samples were incubated at 37*C for 24 hours and sealed using a gas-permeable adhesive film.
2. Remove samples by flicking plate into a tub (to later be bleached as it contains live bacteria). Shake out all the liquid.
3. Wash the plate by submerging in a tub of distilled water (2x). Shake out all the liquid and lightly blot on paper towels to remove excess liquid.
4. Add 0.1% crystal violet and incubate at room temperature for 10-15 minutes.
    - For our assays, we added 200 ul of culture for biofilms. For CV staining, add slightly more than 200 ul to ensure all the adherent biofilm is coated (i.e. 205 ul).
5. Wash plate in tub of distilled water 3-4 times, blotting the plate on paper towels after each wash.
6. Incubate plate upside down at an angle to allow it to fully dry for 2+ hours (no more than 24 hours). After drying, the plates can be imaged to show the visual staining.
7. Add 95% ethanol to the wells (we added 210 ul) and incubate the plate at room temperature for 10-15 minutes.
8. Transfer 150 ul of solubilized CV solution to a new 96-well plate and read the plate at 550 nm.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Weil, A. A., Ng, W. and Chac, D. (2022). Crystal violet biomass assays. Bio-protocol Preprint. bio-protocol.org/prep1751.
2. Barrasso, K., Chac, D., Debela, M. D., Geigel, C., Steenhaut, A., Rivera Seda, A., Dunmire, C. N., Harris, J. B., Larocque, R. C., Midani, F. S., Qadri, F., Yan, J., Weil, A. A. and Ng, W.(2022). Impact of a human gut microbe on *Vibrio cholerae* host colonization through biofilm enhancement. eLIFE. DOI: 10.7554/eLife.73010